Effects of ammonium, phosphate, and salinity on growth, gas exchange characteristics, and ionic contents of seedlings of mangrove *Kandelia candel* (L.) Druce.

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**Abstract.** The effects of ammonium, phosphate, and salinity on growth, gas exchange characteristics, and ionic content of seedlings of mangrove *Kandelia candel* (L.) Druce were investigated in solution culture experiments over seven months. High salinity at 20 ppt NaCl greatly reduced dry matter accumulation in shoots and roots and leaf CO₂ assimilation rate. Addition of ammonium-nitrogen (2 mM) significantly increased the growth of shoots and roots, leaf CO₂ assimilation rate, and respiration rate at low salinity treatments. Phosphate amendment alone did not affect the growth of plants. However, when ammonium-nitrogen was also added, phosphate improved the growth of plants, leaf CO₂ assimilation rate, and respiration rate in low salinity treatments. *Kandelia candel* tended to maintain a constant cation concentration in tissues of leaves and roots at low salinity conditions. Potassium was the major cation in the tissues at the low salinity; however, it was replaced by sodium as the salinity of the culture solution was increased. *Kandelia candel* absorbed ammonium-nitrogen luxuriously at all salinity treatments and some of the ammonium accumulated in the tissues could be part of cations as the osmotic inorganic solute. Reasons for the difference in optimal growth salinity for *K. candel* between porewater in the field and the culture solution in the greenhouse are discussed.

**Keywords:** Ammonium; Growth; *Kandelia candel*; Mangrove; Nitrogen; Nutrients; Phosphate; Salinity.

**Introduction**

The interstitial water and sediment of the stunted mangrove forest in the Chuwei mangrove wetland in Taipei, Taiwan are deficient in both nitrogen and phosphorus (Hwang, 1983; Hwang and Hsu, 1996; Kao and Chang, 1998). A recent field fertilization study indicated that nitrogen is the major nutrient limiting the growth of the stunted mangrove *Kandelia candel* (L.) Druce (Rhizophoraceae) in this wetland (Chen, 1998). The fact that stunted mangrove plants growing in low phosphorus environments did not respond to the addition of phosphorus fertilizer seems unusual, considering results from other mangrove forests in northern Australia (Boto and Wellington, 1983) and in Belize (Feller, 1995). Both of those studies indicated that phosphorus was the major nutrient limiting the growth of *Rhizophora* spp. in the interior, higher elevation areas of the forest.

The availability of essential nutrients, especially phosphorus ion, in waterlogged mangrove soils is largely controlled by the redox potential of the sediment (Patrick and Mahapatra, 1968; Pompanperuma, 1972). Thus, the lack of apparent response to phosphorus fertilization in *K. candel* (Chen, 1998) may indicate that *K. candel* is not phosphorus limited or that the added phosphorus is unavailable for uptake due to precipitation under oxidizing conditions. Additional investigation is required to evaluate these possibilities.

Alternatively, the comparative importance of nitrogen-limitation on the growth of *K. candel* may be related to salinity. Salinity has long been recognized as an important factor regulating growth and distribution of mangroves (Ball, 1988; Lin and Sternberg, 1993; Ball and Pidsley, 1995). Previous greenhouse studies on the growth of *K. candel* seedlings indicated that the optimal salinity for growth was 85 mM NaCl (5 ppt) and that growth was inhibited at salinities above 340 mM NaCl (20 ppt) (Hwang and Chen, 1995). In contrast, the porewater salinity in the Chuwei mangrove swamp was between 25 and 30 ppt (Hwang, 1983; Hwang and Hsu, 1996; Chen, 1998). These observations suggest that the in situ salinity in the Chuwei mangrove swamp may limit the growth of *K. candel*. The accumulation of nitrogenous metabolites in angiosperm halophytes is well documented and may affect the ability of such plants to grow in high salinity conditions (Storey and Wyn Jones, 1975; Popp et al., 1984). Thus the nitrogen fertilization-induced growth increase observed in *K. candel* under high salinity conditions may reflect improved salt tolerance.

The purpose of this study was to examine the growth responses and tissue nutrient contents of *Kandelia candel* to variations in salinity, nitrogen and phosphate under greenhouse culture conditions.
Materials and Methods

**Plant Culture**

Mature propagules of *Kandelia candel* (Druce) were collected from trees growing along Tamsui River, Taipei, Taiwan (121°26'E, 25°9'N). Propagules ranging from 23 to 26 cm in length were used in this study. The bottom 5-cm of each propagule was embedded in a pot of sand (10-cm high, 10-cm diameter). Twenty-four pots were placed in a plastic tub and submerged in culture solution. The experiment was a factorial design with three concentrations of NaCl (0, 85, or 342 mM; or equivalent to 0, 5, or 20 parts per thousand [ppt], respectively) and five of NH\textsubscript{4}\textsuperscript{+}-N (N) and PO\textsubscript{4}\textsuperscript{3-}-P (P) combinations. The five combinations of N and P nutrient solution were NP-11 (0.1 mM N and 0.05 mM P), NP-13 (0.1 mM N and 0.5 mM P), NP-22 (0.5 mM N and 0.1 mM P), NP-31 (2 mM N and 0.05 mM P), and NP-33 (2 mM N and 0.5 mM P) to represent low-N, low-N-high-P, mid-N-P, high-N-low-P, and high-N-P treatments, respectively.

The macronutrients in the basic solution consisted of KCl, CaCl\textsubscript{2}, MgSO\textsubscript{4}, and Fe-EDTA in elemental concentrations (mM) of 6 K, 4 Ca, 10 Mg, 10 SO\textsubscript{4}, and 1 Fe (Epstein, 1972). Salinity, ammonium-N and Phosphate-P were adjusted as needed with NaCl, NH\textsubscript{4}Cl and KH\textsubscript{2}PO\textsubscript{4}, respectively. The pH of the solution was adjusted to 6.5 with CaCO\textsubscript{3} (Asher and Edwards, 1983).

Prior to N and P nutrient treatments, propagules were cultured in the designated salinity (three NaCl solutions) for a month to induce the development of cotyledons and roots. A previous experiment showed that the germination of roots in the first month was greatly affected by the salinity of the culture solutions and not by the N and P nutrients (Hwang and Chen, 1995). Plants growing in similar features were selected for further N and P nutrient treatments. The culture solutions were not aerated and were renewed every month. Tap water was added as required to compensate for losses of solution through evapotranspiration. The concentrations of N and P in the culture solutions were checked every three days and adjusted as needed to maintain initial culture concentrations (Asher and Edwards, 1983). Plants were grown in a greenhouse and were subjected to nutrient and salinity treatments for seven months.

**Growth**

Three plants were harvested from each treatment at 30, 90, 150, and 210 days. Plants were removed from the sand, cleaned in water, rinsed in distilled water, and blotted dry. Plants were separated into leaves, stem, hypocotyl, and roots. Leaf area, stem length, and the fresh weights of all tissues were measured. Plant materials were then dried at 60°C for 1 week for the dry weight measurement. Leaf area was measured with a leaf area meter (Model LI-3000A, LI-COR, Inc., Lincoln, NE, USA). Mean relative growth rate (RGR) was calculated according to RGR = (ln W\textsubscript{2} - ln W\textsubscript{1})/(t\textsubscript{2} - t\textsubscript{1}), where W\textsubscript{1} and W\textsubscript{2} are tissue dry weights at the beginning and end of the experimental period, t\textsubscript{1} and t\textsubscript{2}.

**Gas Exchange Measurements**

Photosynthetic gas exchange was measured on intact leaves with a LCA-4 portable photosynthesis system equipped with a broad leaf type chamber (PLC4(B)) (Analytical Development Company, Herts, England). Four seedlings in each treatment were randomly selected at the end of the experiment, and four mature leaves on the top of the shoots were measured. Plants were placed in a growth chamber for at least one day, and the gas exchange measurement was conducted in the chamber. The environmental conditions in the growth chamber were: light radiation, 600 ± 50 μmol m\textsuperscript{-2} s\textsuperscript{-1}; air temperature, 25 ± 2°C; relative humidity, 60%, and ambient CO\textsubscript{2} concentration. The leaf assimilation rate was recorded at steady state condition, and readings were recorded automatically every 30 s for 2 min. At the end of the measurements, the leaf chamber was covered with a black cloth to evaluate the dark respiration during daylight hours. The conditions and measuring processes for the leaf respiration rate were the same as those for the leaf assimilation rate.

**Tissue Nutrient Analysis**

Tissue nutrient solutions were prepared by the dry ashing method of Kalra and Maynard (1991). Plant materials were dried at 60°C for 1 week to measure the tissue water content. Dry tissues were ground, then ashed at 470°C for 16 h. The ash was further digested in concentrated HCl and HNO\textsubscript{3} solutions and dissolved in dilute acids to bring the minerals into solution. Na and K were analyzed by flame photometry (Ciba Corning Flame Photometer, Model 410, Halstead, Essex, England), Ca and Mg by atomic absorption spectrophotometry (Perkin-Elmer Atomic Absorption Spectrophotometer, Model 2380, Norwalk, Connecticut, USA), and total phosphate determined by the molybdenum blue-ascorbic acid method (Murphy and Riley, 1962). A CHN analyzer (NA 1500 series 2, Fisons Instruments, Italy) was used to determine the total nitrogen content of the sample. We expressed ion concentrations on a tissue water basis to reflect the activities of ions in tissues.

Mean values of replicates of each parameter were compared for all salinity and nutrient treatments using the SAS general linear model procedure (PROC GLM) two-way ANOVA and Tukey's Studentized Range test (P ≤ 0.05; SAS, 1989).

**Results**

The differences in growth parameters were similar for each treatment during different harvesting stages (data not shown). Therefore, only results collected at the end of the experiment (210 days) are presented.

**Growth**

Salinity and N significantly impacted the growth of *K. candel*, and there was a significant interaction between salinity and nutrient level (Figure 1). At 0 and 5 ppt
salinity, high concentration of N (NP-31 and NP-33) greatly increased the growth of aboveground tissues; e.g., leaf area (Figure 2A), leaf dry weight (Figure 2B), stem height (Figure 2C), and stem dry weight (Figure 2D). High salinity (20 ppt) had an overall detrimental effect on the growth of K. candel (Figures 1-3), and adding N or P nutrients did not relieve the effect. The effect on growth of high P alone (NP-13) did not differ significantly from low P at 0 and 5 ppt salinity. However, P improved the growth in high N condition (NP-33), and the improvement was higher at 5 ppt than at 0 ppt salinity (Figures 1-3). Apparently, N is the prime limiting nutrient for the growth of K. candel at low salinity conditions and some salinity is beneficial to its growth. The growth of roots was mainly affected by salinity. High salinity inhibited growth (Figure 3). Except for the NP-33 treatment, no significant difference in root growth was found among nutrient level treatments for each salinity treatment. The changes in weight and length of hypocotyl were not significant among treatments during the experiment (data not shown).

**Gas Exchange Measurement**

Net CO$_2$ assimilation rate of K. candel also was mainly affected by N and salinity (Figure 4A). High N treatments (NP-31 and NP-33) only increased net CO$_2$ assimilation rate in low salinity treatments (Figure 4A). High salinity (20 ppt) reduced net CO$_2$ assimilation rate.

The leaf respiration rate was mainly affected by the nutrient level treatments. High NP treatments significantly increased the leaf respiration rate, but high P treatment alone (NP-13) significantly reduced the leaf respiration rate (Figure 4B). The salinity levels used in this experiment did not significantly affect leaf respiration rate (Figure 4B).

![Figure 2](image2.png)

**Figure 2.** Effects on Kandelia candel of different salinity and NP nutrient conditions on (A) leaf area per plant, (B) leaf dry weight per plant, (C) stem length per plant, and (D) stem dry weight per plant. Bars represent LSD ($P=0.05$, $n=3$).

![Figure 1](image1.png)

**Figure 1.** Relative growth rate in Kandelia candel grown under different salinity and NP nutrient conditions. Bars represent LSD ($P=0.05$, $n=3$).

![Figure 3](image3.png)

**Figure 3.** Effects on Kandelia candel of different salinity and NP nutrient conditions on root dry weight per plant. Bars represent LSD ($P=0.05$, $n=3$).
Inorganic Solute

Salinity greatly affected the concentration of cations in tissues of *K. candel*. The tissue-Na-contents increased significantly with increasing salinity in culture solutions (Figure 7), and neither N nor P levels (Figure 7) affected this increase. In contrast, the tissue contents of K (Figure 8), Ca (Figure 9), and Mg (Figure 10) all decreased significantly in 20-ppt NaCl salinity. Apparently, Na replaced these inorganic solutes in tissues of *K. candel* at high salinity. Moreover, there was a high correlation between solution Na contents and the leaf tissue Na contents. Where,

\[
\text{Leaf-Na (mM)} = \text{Solution-Na (mM)} \times 1.0618 + 46.608, \\
\text{r}^2 = 0.9776.
\]

Potassium was the dominant inorganic cation in shoots of *K. candel* at low salinities (Figure 8). However, in the leaf tissues there was a reduction of K contents, not only with increasing salinity, but also with increasing N levels (Figure 8). A similar phenomenon was true for tissue-Ca and Mg-contents. Ca (Figure 9) and Mg (Figure 10) contents in shoots (leaf and stem) were not only reduced by salinity, but also by high N levels.

In roots, the contents of Mg (Figure 9) and Ca (Figure 10) were inversely related to salinity, and not affected by N or P levels. Very low Ca concentrations were observed in roots, compared with the shoot (Figure 9), in all treatments.

Figure 4. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on (A) leaf assimilation rate and (B) leaf respiration rate. Bars represent LSD (P=0.05, n=4).

Tissue Nutrient Contents

**Nitrogen.** N had significant effects on the tissue-N-contents in *K. candel* (Figure 5). The concentration of tissue-N was higher in shoots than in roots and higher in leaves than in stems. High N culture solutions resulted in higher tissue-N-contents. The N contents in leaf tissues in NP-31 and NP-33 treatments could exceed 500 mM (ca 3% based on leaf dry weight), significantly higher than for lower N treatments in each salinity treatment.

The tissue-N-contents were not significantly affected by salinity treatments (Figure 5). High salinity restricted the growth of *K. candel* (Figure 1) but did not inhibit the accumulation of nitrogen in tissues (Figure 5), suggesting luxury consumption of N by *K. candel*.

**Phosphate**

P had greater effects on root-P-contents than leaf-P-contents. High P in combination with high N (NP-33) solutions increased root-P-contents, and this effect was inversely related to salinity treatments (Figure 6). However, this phenomenon was not found in leaf tissues. Salinity did not significantly affect the leaf-P-contents (Figure 6).

Increasing P alone (NP-13) only slightly increased the leaf- and root-P-contents in *K. candel* (Figure 6), but this did not result in a growth increase (Figure 1). Plants with high-N-low-P treatments (NP-31) had lower tissue-P-contents than controls (NP-11) (Figure 6), which was probably caused by the dilution effect due to N stimulating growth.

Figure 5. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on nitrogen contents in leaf, stem, hypocotyl and root tissues. Bars represent LSD (P=0.05, n=3).
Figure 6. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on phosphorus contents in leaf, stem, hypocotyl and root tissues. Bars represent LSD (*P*<0.05, *n*=3).

Figure 7. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on sodium contents in leaf, stem, hypocotyl and root tissues. Bars represent LSD (*P*<0.05, *n*=3).

Figure 8. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on potassium contents in leaf, stem, hypocotyl and root tissues. Bars represent LSD (*P*<0.05, *n*=3).

Figure 9. Effects on *Kandelia candel* of different salinity and NP nutrient conditions on calcium contents in leaf, stem, hypocotyl and root tissues. Bars represent LSD (*P*<0.05, *n*=3).
Kandelia candel was able to regulate and accumulate high cation contents in tissues even grown at low salinity. When the concentration of major cations (K, Na, Ca and Mg) and nitrogen were summed, we found that K. candel seedlings maintained a cation concentration of 800 - 1000 mM in leaves and ca 400 mM in roots (Figure 11) and that there was no significant difference among nutrient level treatments in low salinity conditions. However, plants grown in 20 ppt NaCl salinity generally had a significantly higher tissue cation content than those in lower salinity (Figure 11).

Discussion

This study demonstrated that salinity was the primary factor affecting the growth of Kandelia candel. High salinity at 20 ppt significantly reduced the growth (Figures 1-3) and net CO_2 assimilation rate (Figure 4A), and the effects were not relieved by the addition of high concentrations of nitrogen and phosphate. However, NaCl improved growth and net CO_2 assimilation rate at low salinity (Figures 1-4A). Plants cultured in 5 ppt NaCl salinity displayed greater growth and a higher net CO_2 assimilation rate than those at 0 ppt NaCl. Moreover, high concentrations of N alone in the culture solution greatly increased growth and net CO_2 assimilation rate at low salinity, but P alone did not. Such growth enhancement in response to added N at low salinities, but not at higher salinities, has been commonly observed in other mangrove studies (Downton, 1982; Clough, 1984; Boto et al., 1985; Naidoo, 1987, 1990; Ball and Pidsley, 1995).

These results are consistent with the results of field fertilization experiment (Chen, 1998). The field fertilization experiment conducted at the Chiwei mangrove swamp indicated that the growth of K. candel was limited by the nitrogen availability, but not by phosphate. Adding P will enhance growth and net CO_2 assimilation rate only in high N conditions. Apparently, the growth of K. candel mainly responds to nitrogen not phosphate when salinity is not the limiting factor. This is in contrast to the findings of Boto and Wellington (1983) and Feller (1995) of phosphorus deficient dwarfism observed in mangrove forests at higher elevations.

Kandelia candel can accumulate a high concentration of cations in tissues, even when cultured without NaCl (Figure 11). This phenomenon was previously reported in other mangrove species (Atkinson et al., 1967; Downton, 1982; Clough, 1984; Ball et al., 1987; Naidoo, 1987) and was suggested to serve an osmoregulatory function in saline environments (Flowers et al., 1986; Ball, 1988). Kandelia candel maintained cation levels below 400 mM in leaf tissues (if the N contents were not included) at low salinities. Potassium was the major ion in the tissues of K. candel at low salinities (Figure 8), but was replaced by...
sodium (Figure 7) as the concentration of NaCl in the rhizosphere was increased. It has been suggested that Na can interfere with K absorption in roots (Munn et al., 1983). Ball et al. (1987) suggested that the deficiency of K in the tissues of mangroves grown at high NaCl salinity could cause the reduction of photosynthetic rate (Figure 4A).

The results of this study indicated that the growth inhibition observed in *K. candel* at high salinities was not relieved by N addition; however, NH₄⁺ may contribute to osmoregulation in *K. candel*. *Kandelia candel* cultured in high N conditions accumulated high concentrations of N (Figure 5) while the concentrations of K, Mg, and Ca in the tissues were all greatly reduced (Figures 8-10).

The optimal salinity for *K. candel* grown in the greenhouse was 5 ppt NaCl, and growth was inhibited at 20 ppt (Hwang and Chen, 1995). In the field, however, *K. candel* exhibits luxurious growth even at porewater salinities ranging from 15 to 30 ppt (Table 1). Similar discrepancies in the optimal growth salinity of mangroves grown in the field versus the greenhouse have been reported previously. The optimal growth salinities reported for mangroves in the greenhouse range from 5% (ca 1.5 ppt) to 50% (ca 15 ppt) seawater (Downton, 1982; Clough, 1984; Boto et al., 1985; Naidoo, 1987; Ball, 1988; Naidoo, 1990; Ball and Pidsley, 1995). In the field, these same mangrove species grow well at salinities ranging from 50% up to over 100% seawater.

Differences in freshwater supply are probably responsible for the apparent discrepancy in optimal growth salinity. While greenhouse plants were continuously exposed to constant salinity conditions, in situ plants are routinely inundated with relatively fresh water, as well as higher salinity porewater. Mangroves growing in high saline environments preferentially take up fresh water when available. For example, stable hydrogen and oxygen isotope analyses indicated that the dwarf red mangrove, *Rhizophora mangle L.*, preferentially utilized rain-derived freshwater during the wet season (Lin and Sternberg, 1992). Similarly, mangrove plants in the Chwui wetland may preferentially take up water during the relatively fresh initial stage of tidal inundation. The salinity of the initial inundating water in the Chwui wetland is about 15 ppt compared to 30 ppt at high slack tide (Chen, 1998). Results of the present greenhouse study indicated that the Na contents in the leaves of *K. candel* are closely related to the Na concentration of the culture solution. Thus, based on the tissue Na contents of *K. candel* growing in situ, the salinity of the uptake water is probably 6 to 14 ppt salinities for *K. candel* growing along the Tamsui River in Taipei (Table 1) even though the porewater salinity ranges from 10 to 32 ppt (Table 1).

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**Literature Cited**


Hwang, Y.-H. 1983. Dynamics of Nutrient Flow in the Chuwei Mangrove Ecosystem. MS thesis, Department of Botany, National Taiwan University, Taipei, Taiwan.


Hwang, Y.-H. and M.-C. Hsu. 1996. Study on the growth limiting factors in the Chuwei dwarf mangrove, Taiwan. In Symposium on the Mangrove Ecosystem, held by Taiwan Endemic Species Research Institute on Dec. 29, 1995. Taichung, Taiwan, ROC, pp. 3-20.


氨態氮、磷酸鹽和鹽度影響水筆仔紅樹林幼苗的生長、氣體交換和組織離子含量等特性之研究

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我們觀察水筆仔紅樹林幼苗在溫室水耕試驗中，其生長、氣體交換和組織離子含量等特徵受水耕溶液中氨態氮、磷酸鹽和鹽度變化的影響。在 20 ppt NaCl 鹽度下，植株全株的生長速率和葉片 CO₂ 交換速率嚴重受到抑制。在低鹽度下，高氨態氮 (2 mM) 顯著提高植株的生長速率，葉片的 CO₂ 同化速率和呼吸速率。若只增加磷酸鹽含量則不影響植株的生長。但是增加磷酸鹽可提升原本受氨態氮刺激的植株生長速率，葉片 CO₂ 同化速率和呼吸速率。在低鹽度下，水筆仔葉片和根部組織可維持一定濃度的陽離子總量。在 0 ppt NaCl 鹽度下，鉀離子是組織中陽離子的主要成份；但隨著水耕溶液中 NaCl 含量增加，鈉離子取代鉀離子成為組織中陽離子的主要成份。在各種鹽度試驗中，水筆仔紅樹林幼苗對水耕溶液中氨態氮的吸取均呈現「奢侈性吸收」的現象，而其中部分氨態氮可能成為組織內調控體內無機溶質的一部分。本文中亦討論造成水筆仔紅樹林在溫室水耕和野外自然環境中生長差異之可能原因。

關鍵詞：生長；水筆仔；紅樹林；營養鹽；氨態氮；磷酸鹽；鹽度。